



Assessment of diversity by using morphological, biochemical and molecular approaches of selected basmati rice (*Oryza sativa* L.) varieties

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Received: March 29, 2015; Revised received: October 19, 2015; Accepted: January 24, 2016

Abstract: The study investigates the genetic diversity among the Basmati rice genotypes. Selected nine Basmati rice genotypes were studied for twelve morphological traits, biochemical parameters and for molecular analysis with 11 SSR markers. Pusa Sugandha 5 and Basmati 370, showed strong aroma while other varieties showed medium aroma. Alkali spreading value were intermediate in Basmati 386, Vallabh Basmati 22 and Vallabh Basmati 24 while other varieties showed high values. Pusa Basmati 1 and Basmati 386 showed soft category of gel consistency while in rest varieties it was under medium category. Amylose percentage in grains were ranged from 18.02% (Taraori basmati) to 22.0% (Basmati370). Molecular analysis with 11 SSR markers showed 125 alleles with an average number of alleles 11.36 per locus. All the markers showed specific type of banding pattern along with 82 polymorphic alleles in different genotypes. This study focuses on application of statistical methods and techniques in analysis of genetic diversity of the agronomic data, biochemical aspects related to quality parameters and at the molecular level using SSR markers for clustering procedure making dendrogram that helps the more accurate selection of the superior basmati genotypes for the further studies of the breeders and researchers.

Keywords: Amylose, Aroma, ASV Basmati rice, Diversity, GC and SSR Primers

INTRODUCTION

Basmati rice is premium rice with high aroma and grain quality. After cooking Basmati rice length increases more than twice of its original length. The aroma and its sweet taste make it delicious. Grain and cooking quality analysis of Basmati rice reveals large variation in grain dimensions, brown rice colour, texture of cooked rice, intensity and kind of aroma and adaptation to certain agro-climatic conditions. Grains with similar amylose content, gel consistency and alkali spreading value also show variable cooking behaviours. The molecular characterization is a necessary step to determine whether any germplasm is a part of the gene pool of the system concerned. This is especially important in cases where morphological markers can prove to be inaccurate. Molecular markers like RAPD, SSR and ISSR have been used for the genetic diversity in different crops. These markers are extremely sensitive and are capable of identifying allelic variability in early stage of plant growth. SSR (Simple Sequence Repeat) markers or microsatellites are tandem repeats interspersed throughout the genome and can be amplified using primers that flank these regions (Grist *et al.*, 1993). The assessment of diversity of In-

dian rice genotypes has been carried out by many researchers in the past (Saini *et al.*, 2004; Ram *et al.*, 2007; Sundaram *et al.*, 2008, Kumar *et al.*, 2010; Sivarajani *et al.*, 2010 and Vanniarajan *et al.*, 2012). There are few reports of genetic diversity analysis of basmati rice of Indian continent (Nagaraju *et al.*, 2002). We planned this study to work out the diversity assessment of popular basmati rice varieties of India.

MATERIALS AND METHODS

The Basmati rice varieties were grown in season 2012 to 2013 at the Crop Research Centre and other laboratory works were conducted at, department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut (U.P.) at latitude of 29° 40' North and longitude of 77° 42' East and at an altitude of 237 meter above mean sea level.

Evaluation of agronomic trait: Data were recorded on five competitive plants per genotype from the middle row for yield and yield components such as days to flower (DF), days to maturity (DM), plant height (PLH), number of tillers per plant (TLN), panicle length (PNL), panicle bearing tillers per plant (PBT), spikelet fertility percentage (SFP), leaf area (LA), Test

weight (TW), Biological yield (BY), Harvest index (HI) and Grain yield per plant (GYP).

Evaluation of biochemical traits (quality traits)

Aroma test : Take 5 gm of rice soaked in 15 ml of water for 10 min and cook for 15 min. Cooked rice transferred into a petri dish and placed in refrigerator for 20 min for cooling. The cooked rice was evaluated by olfactory method with as, a random panel; strongly scented (SS); mild scented (MS); non scented (NS) (Anonymous, 2004).

Alkali spreading value (ASV) and Clearing test: Six milled rice were taken in Petri plates and 10 ml of potassium hydroxide (0.34 M KOH) was added to the samples, Samples were kept for 23 hours in an incubator at 27-30 °C (Perez and Juliano, 1978)

Amylose content (AC): Take 100 mg sample of rice flour 1 ml of 95% ethanol and 9 ml of 1.0 N NaOH were mixed and heated in a boiling water-bath for 10 min. Samples were diluted to 100 ml with distilled water. Take 5 ml diluted sample and 1 ml of acetic acid (57.75 ml Glacial acetic acid in one litre water) was added to acidify the sample along with 1.5 ml of iodine solution (0.2% iodine + 2% potassium iodide). Sample of volume was made upto 100 ml with distilled water. The samples were incubated at room temperature for 20 min. The absorbance was measured at 620 nm using spectrophotometer. As a control, NaOH solution were used. The AC of different varieties was calculated in comparison with standard graph (Perez and Juliano. 1978).

Gel consistency (GC): Take 100 mg sample of rice flour in test tube (2×19.5 cm), add 0.2 ml of ethanol containing 0.25% thymol blue and 2.0 ml of 0.2 N of KOH were added and kept in boiling water-bath for 8 min. Cooled, mixed well and kept in ice bath for 20 min. Later the test tubes were laid horizontally for one hour and measurements were made using graph paper. The degree of disintegration of kernel was evaluated using a 7 point scale (Bhattacharya. 1979).

Selection of SSR markers: A prior information was used to select random as well as trait-linked SSR markers. Randomly selected 11 markers having wide genome distribution that could effectively illustrate the diversity among the genotypes, developed by the Cornell University, Ithaca (Mc Couch *et al.*, 2002).

Marker assay: Total genomic DNA isolation was carried out by a CTAB method (Murray and Thompson. 1980) and assayed with a total of 11 SSR markers. The PCR reactions were carried out in sterile 0.2 ml thin-walled PCR tubes. Amplification was carried out in 20 µl reaction volume (25-50 ng DNA template 1 µl, 10 mM dNTP mixture 0.6 µl, 1 U/µl Taq DNA polymerase 0.5 µl. PCR Reaction buffer 2.0 µl, Primer F 1.0 µl, Primer R 1.0 µl, Double Distilled water 13.5 µl. The PCR amplification was done in a thermo-cycler which was programmed as follow denatured at 94°C for 3 minutes followed by 35 cycles (1 min denaturation at 94°C, 1 minutes annealing at 55°C and 1 min of primer extension at 72°C) of PCR amplification followed by final extension of 72°C for 7 minutes and

4°C for forever Storage. The amplification products were separated on 2% agarose gels and run for 2 h in 1X TAE buffer. DNA fragments were visualized under UV transilluminator using Alpha Imager gel doc.

Data analysis: The phenotypic data were subjected to statistical analysis for descriptive purposes, as well as for character association. ANOVA analysis using software OPSTAT. The dendrogram was constructed using the software package NTSYSpc (Numerical Taxonomy System). Amplicons were scored for their product size for each primer genotype combination. The informativeness of the markers in terms of polymorphism was determined by calculating the polymorphism information content (PIC) (Botstein *et al.*, 1980) for each SSR locus, according to the formula,

$$PIC = 1 - \sum p_i^2$$

Where p_i is the frequency of the i th allele (Smith *et al.*, 1997)

RESULTS AND DISCUSSION

Agronomic traits: Morphological traits such as DF, DM, PLH, PL, TLN, PBT, SFP, LA, BY, TW, HI and GYP under study showed wide range of variation in different rice genotypes. Flowering date (DF) ranged from lowest for Basmati 370 (97.38 days) to highest for Type3 (130.05 days). Days of maturity (DM) was recorded minimum for Pusa Sugandha 4 (120 days) to maximum for Basmati 386 (156.33 days). The plant height (PLH) ranged from minimum for Pusa Basmati 1 (85.68 cm) and maximum for Basmati 370 (153.23 cm). Plant height are highly diverse in different rice varieties (Tahir *et al.*, 2002 and Golam *et al.*, 2011). Tillers numbers per plant (TLN) was recorded minimum for Pusa Sugandha 5 (10.40 tillers) to maximum for Basmati 370 (20.13 tillers) tillers per plant. Panicle bearing tillers per plant (PBT) ranged from Pusa sugandha 5 for minimum is 8.69 to maximum for Basmati 370 is 18, The panicle length (PNL) of plant ranged from minimum is Pusa sugandha 4 is 28.41 cm to maximum for Basmati 370 is 34.18 cm. the spikelet fertility percentage (SFP) is ranged from Taraori Basmati (94.47%) to highest for Basmati 370 (97.70 %). The leaf area (LA) was recorded 35.98 cm minimum for Vallabh Basmati 22 to 65.68 cm maximum for Basmati 370. The biological yield (BY) ranged from minimum for (Pusa Basmati 1) to maximum for Basmati 386 (167.66 q/ha). Test weight is ranged from Basmati 370 (21.26 gm), to Pusa sugandha 4 (28.43 gm). The grain yield (GY) ranged from minimum for Type 3 (33.32 q/ha) to maximum for Vallabh Basmati 24 (64.21 q/ha). The Harvest index (HI) was observed minimum for Type 3 (23.32 %) to maximum for Vallabh Basmati 24 (55.25 %). The mean performances of nine genotypes for twelve characters are given in Table 1. All the varieties showed the different pattern in morphological characters.

Biochemical Traits (Quality traits)

Aroma test: Basmati 370 and Pusa Sugandha 5 showed the presence of strong aroma. Type 3, Pusa

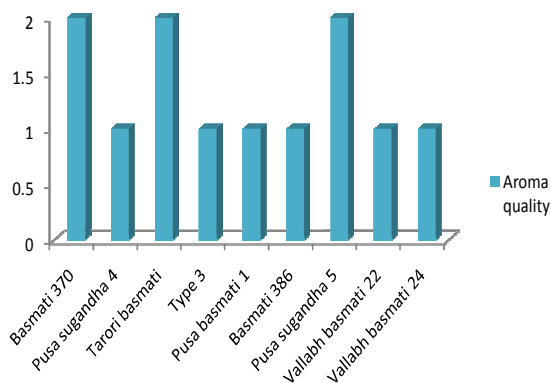


Fig.1. Aroma test for nine selected rice varieties.

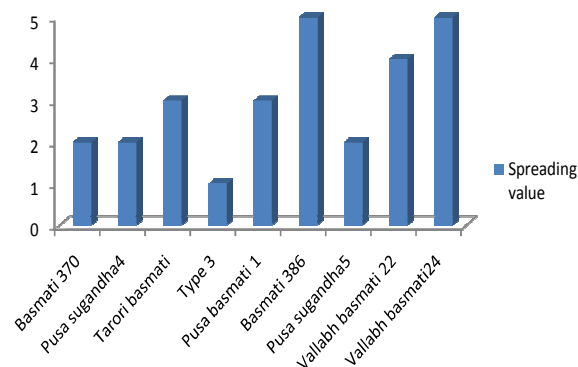


Fig. 2. Alkali spreading value (ASV) of nine selected rice varieties.

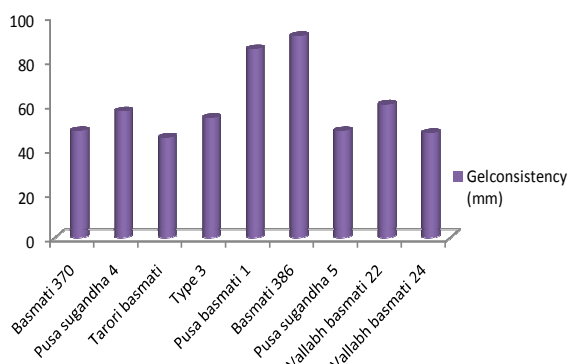


Fig. 3. Gel consistency (GC) of nine selected rice varieties.

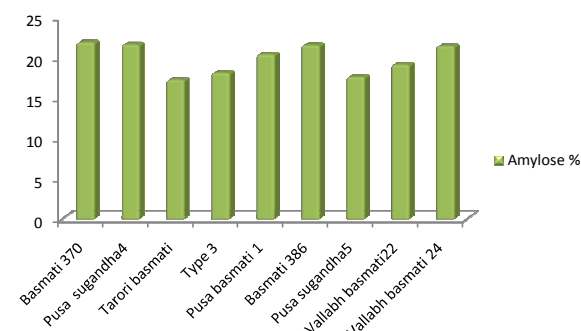


Fig. 4. Amylose percentage of nine elected varieties.

Basmati 1, Basmati 386, Vallabh Basmati 22 and Vallabh Basmati 24 showed presence of mild aroma (Fig.1). Pusa Basmati 1 and Pusa sugandha 5 contains more aroma than other traditionally cultivated scented rice varieties (Nadaf *et al.*, 2006).

Alkali Spreading Value and Gelatinization: Alkali spreading value were recorded as high, intermediate and low. Alkali Spreading Value (ASV) and Gelatinization test (GT) (Table 2, Fig. 2) revealed that low ASV (1-3) and high GT were detected in Type 3, Basmati 370, Pusa Sugandha 4, Taraori Basmati, Pusa Basmati 1, and Pusa Sugandha 5. The intermediate alkali spreading value and gelatinization were observed in basmati 386, Vallabh Basmati 22, and Vallabh Basmati 24 varieties. The traditional rice varieties shows low ASV and high gelatinization temperature which is highly desirable for the grain quality (Bansal *et al.*, 2006). Gelatinization is the process in which the intermolecular bonds of starch molecules are broken down in the presence of water and heat, thereby dissolving the starch granules in the endosperm. The gelatinization temperature determines the time required for cooking.

Gel consistency: Gel consistency (GC) were measured as soft, medium, and hard among basmati rice varieties. Gel consistency ranged from 65 to 100 mm as soft, 41 to 64 mm as medium and 26 to 40 mm hard.

The length of the blue gel was maximum (91mm) in Basmati 386 and followed by (85 mm) Pusa Basmati. The varieties Pusa Sugandha 5, Vallabh Basmati and Vallabh Basmati 24 recorded 48 mm, 22mm, 60 mm and 47 mm length of blue gel respectively and categorized under medium gel consistency. The lowest GC was recorded in Taraori Basmati (45mm) (Table 3, Fig.3). Gel consistency refers as a parameter to index the tendency of cooked rice under soft to hard on cooling and is normally classified as hard, medium, and soft (Chen *et al.*, 2012).

Amylose content: Rice varieties are grouped on the basis of their AC into waxy (0-2%), very low (3-9%), low (10-19%), intermediate (20-25%), and high (>25%) (Shahidullah *et al.*, 2009). Low amylose content was observed in Taraori Basmati (18.02 %), Type 3 (18.57 %) and Pusa Sugandha 5 (18.86 %). The amylose content was under intermediate range in 19.80% Vallabh Basmati 22 to 22.0% (Basmati 370). High amylose content was recorded in Basmati 370 (22.00 %), followed by Pusa Sugandha 4 (21.40 %), Pusa basmati 1 (20.56 %), Basmati 386 (21.70 %) and Vallabh Basmati 24 (21.64%) (Fig.4). Most of the traditional aromatic varieties showed low amylose content in comparison with evolved Basmati rice varieties. Cooking quality is mainly determined by the content and composition of starch (amylose and amy-

Table. 1: Agronomical data of nine selected Basmati rice varieties.

S.No.	DF	DM	PLH	PNL	TLN	PBT	SFP	LA	TW	BY	GYP	HI
	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
1	97.38 \pm 1.47	150.66 \pm 0.66	153.28 \pm 3.64	34.18 \pm 0.93	20.13 \pm 0.48	18.20 \pm 0.30	97.70 \pm 0.30	65.68 \pm 2.304	21.26 \pm 0.73	143.33 \pm 0.88	55.35 \pm 1.45	38.57 \pm 0.76
2	101.40 \pm 2.00	120.60 \pm 0.37	105.43 \pm 0.56	28.41 \pm 0.04	17.26 \pm 0.48	15.48 \pm 1.37	94.98 \pm 0.76	38.29 \pm 1.19	28.43 \pm 0.75	138.00 \pm 10.59	48.97 \pm 4.92	36.84 \pm 0.53
3	119.62 \pm 1.93	155.33 \pm 0.66	142.56 \pm 1.09	29.96 \pm 0.31	13.61 \pm 0.81	12.85 \pm 0.37	96.00 \pm 0.38	36.35 \pm 0.52	25.26 \pm 0.60	135.76 \pm 2.38	49.74 \pm 2.14	37.19 \pm 0.90
4	130.05 \pm 0.38	147.33 \pm 0.66	140.86 \pm 2.60	32.14 \pm 0.38	13.52 \pm 0.06	12.29 \pm 0.12	97.60 \pm 0.64	64.00 \pm 0.46	22.28 \pm 0.08	142.33 \pm 3.38	33.32 \pm 1.65	23.26 \pm 0.83
5	109.74 \pm 2.22	134.00 \pm 1.00	85.68 \pm 0.63	29.36 \pm 0.57	13.20 \pm 0.80	11.05 \pm 0.77	97.11 \pm 0.46	56.66 \pm 0.645	23.89 \pm 0.25	93.78 \pm 0.77	45.23 \pm 1.48	49.01 \pm 0.89
6	122.83 \pm 1.46	156.33 \pm 0.66	110.13 \pm 0.90	31.92 \pm 0.33	10.64 \pm 0.34	9.50 \pm 0.07	94.78 \pm 0.74	48.87 \pm 0.756	24.08 \pm 0.07	167.66 \pm 1.45	56.55 \pm 4.12	35.63 \pm 1.32
7	97.60 \pm 0.87	121.33 \pm 1.33	104.04 \pm 2.95	30.64 \pm 0.45	10.40 \pm 0.75	8.69 \pm 0.65	94.47 \pm 0.49	40.72 \pm 3.90	24.73 \pm 0.09	99.33 \pm 1.20	43.88 \pm 2.07	43.61 \pm 1.26
8	120.81 \pm 0.83	141.33 \pm 0.66	100.91 \pm 2.10	28.41 \pm 0.79	11.67 \pm 0.26	10.32 \pm 0.05	97.49 \pm 0.46	35.98 \pm 1.09	22.62 \pm 0.37	131.46 \pm 3.25	40.97 \pm 3.82	32.98 \pm 1.55
9	126.35 \pm 0.56	150.66 \pm 0.66	132.95 \pm 1.46	33.45 \pm 0.45	15.14 \pm 0.29	15.00 \pm 0.83	97.76 \pm 0.58	42.38 \pm 1.25	22.52 \pm 0.27	132.13 \pm 4.50	64.21 \pm 3.27	45.25 \pm 0.97
CD	4.041	2.516	6.285	1.719	1.856	2.013	1.697	4.724	1.261	13.497	8.222	2.730
SE(D)	1.890	1.177	2.939	0.804	0.868	0.941	0.794	2.209	0.590	6.313	3.845	1.277
SE(M)	1.337	0.832	2.078	0.569	0.614	0.666	0.561	1.562	0.417	4.464	2.719	0.903
CV	2.031	1.015	3.011	3.182	7.619	9.150	1.005	5.677	3.022	5.878	9.671	3.995

(1 - Basmati 370, 2- Pusasugandha 4, 3- Tarori Basmati, 4- Type 3, 5- Pusa Basmati 1, 6- Basmati 386, 7- Pusasugandha 5, Vallabh Basmati 22, 9- Vallabh Basmati 24.)
 DF, Date of flowering., DM, Date of maturity., PLH, Plant height(cm), PNL, Panicle length(cm), TLN, No. of tillers., PBT, Panicle bearing tillers., SFP, Spikelet fertility percentage.,
 LA, Leaf area (cm), TW, Test weight (gm), BY, Biological yield (q/ha), HI, Harvest Index (%), GY, Grain yield (q/ha).

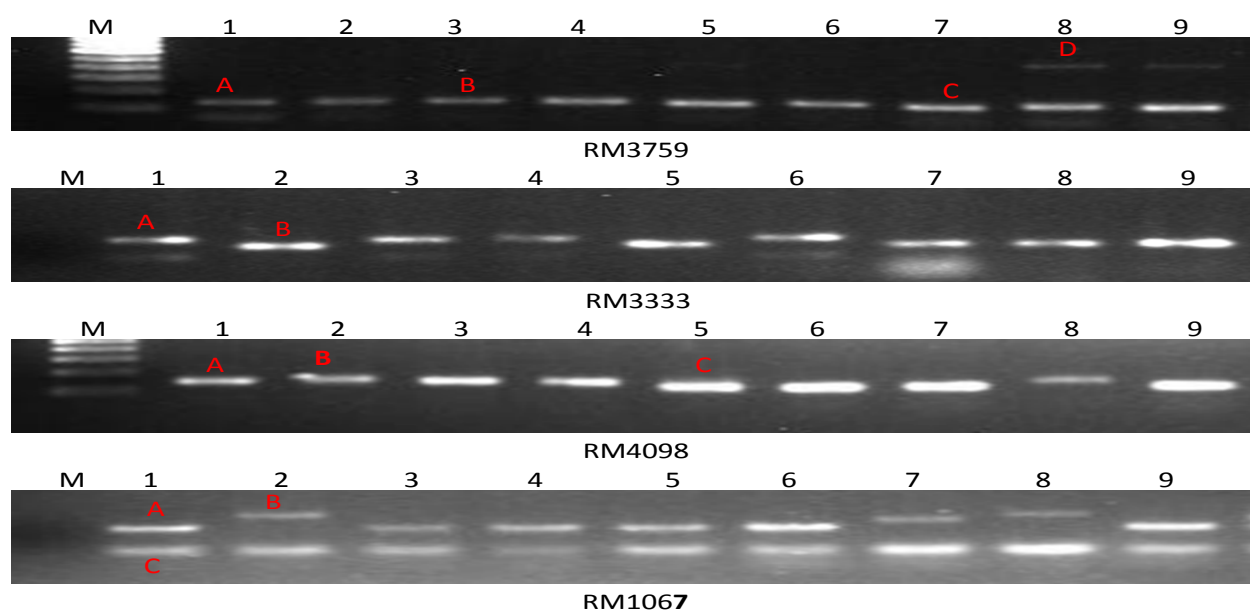


Fig. 5. A representative gel picture showing allelic diversity for different SSR markers; M, 100-bp ladder; 1–9, rice germplasm lines; A, B, C, and D, different alleles amplified.

lopectin) and storage protein in the endosperm are the main factors (He and Suzuki. 1987; Sowbhagya *et al.*, 1987; Rani and Bhattacharya. 1989; Ong and Blanchard. 1995; Singh *et al.*, 2003; Cameron and Wang 2005; Allahgholipour *et al.*, 2006; Kibanda and Luzi-kihupi. 2007). Amylose content and the chain structure of amylopectin were found to be significantly correlated with hardness and stickiness of the rice after cooking (Ramesh *et al.*, 1999; Mizukami and Takeda 2000; Nakamura and Juliano. 2006). The amylose content in all grades of rice ranges between 20.7- 21.4%. After cooking rice become moist and sticky due to low amylase content (Shahidullah *et al.*, 2009). High amy-

lose grains cook dry, are less tender, and become hard upon cooling. In contrast, low-amylose rice cooks moist and sticky. Intermediate amylose rice is preferred in most rice growing areas of the world (Randall *et al.*, 2009).

Molecular analysis: DNA primers have the ability to differentiate different genotype based on the difference in the representing genomic regions and also depend upon the number of alleles. Out of eleven, six SSR markers were found to be polymorphic (Fig. 5). The average PIC of these markers was 0.36 exhibiting the highest PIC 0.74. The average number of polymorphic alleles is 3.67, amplified by SSR Primers led to the

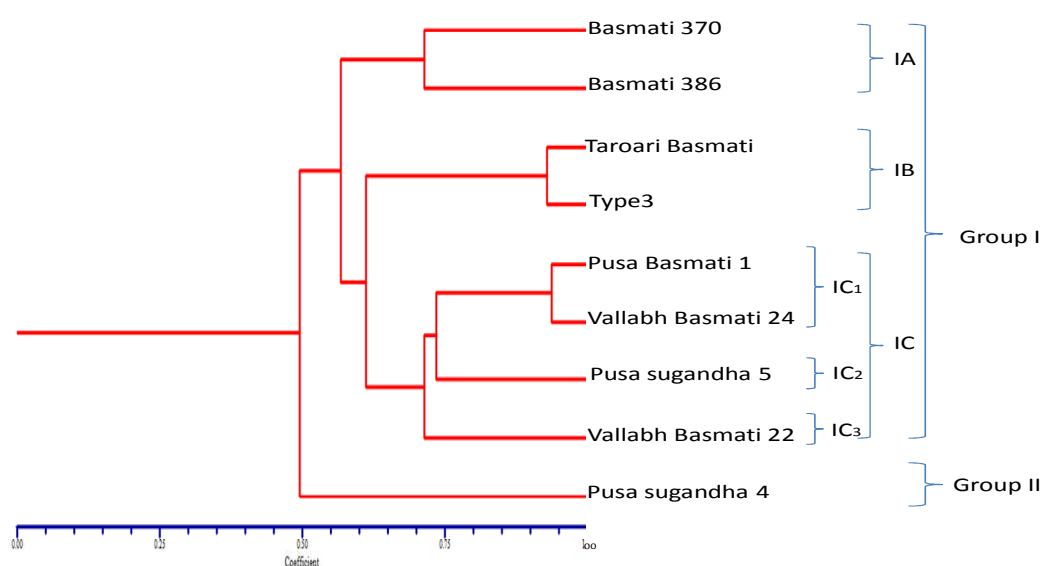


Fig.6. Molecular dendrogram showing the genetic similarity among aromatic rice varieties.

Table. 2: Biochemical parameters (Quality parameters) of selected Basmati rice varieties.

S.No.	VARIETY	AROMA	ALKALI SPREADING VALUE		AMYLOSE PERCENTAGE		GEL CONSISTENCY	
			SPV	GT	CATEGORY	AMY %	CATEGORY	GC (mm)
1	Basmati 370	Strong aroma	2	75-79	High	22.00	Intermediate	48
2	Pusa sugandha 4	Mild aroma	2	75-79	High	21.40	Intermediate	57
3	Taroari Basmati	Strong aroma	3	75-79	High	18.02	Low	45
4	Type 3 Basmati	Mild aroma	1	75-79	High	18.57	Low	54
5	Pusa Basmati 1	Mild aroma	3	75-79	High	20.56	Intermediate	85
6	Basmati 386	Mild aroma	5	70-74	Intermediate	21.51	Intermediate	91
7	Pusa sugandha 5	Strong aroma	2	75-79	High	18.86	Low	48
8	Vallabh Basmati 22	Mild aroma	4	70-74	Intermediate	19.80	Low	60
9	Vallabh Basmati 24	Mild aroma	5	70-74	Intermediate	21.402	Intermediate	47

amplification of total 125 bands, out of which 82 bands are polymorphic and while 43 bands were monomorphic. Primer RM 4469 and RM 1067 amplify maximum number of bands and RM4405 and RM1003 amplified minimum number of bands. On an average 11.36 bands amplified per primer. The primers pair RM4469 and RM1067 with an average number of PIC value 0.97 is maximum for SSR primer. All nine cultivars are grouped (Fig. 7) into two major groups (Group I and Group II) based upon the Multivariate analysis of genetic similarity data. At the coefficient $J = 0.50$ Group I consist of eight genotype, Basmati 370, Basmati 386, Taroari Basmati, Type 3, Pusa Basmati 1, Vallabh Basmati 24, Pusa sugandha 5 and Vallabh Basmati 22 whereas Group II consist of one genotype, Pusa Sugandha 4. Group I is further divide into three sub groups (IA, IB and IC). At the coefficient of $J = 0.55$ to 0.60 subgroup IA consist of two genotypes, Basmati 370 and Basmati 386 at the coefficient $J = 0.70$. Group IB also consist of two genotypes, Taroari Basmati and Type 3 at the Coefficient $J = 0.94$. Whereas subgroup IC further divided in three sub group (IC₁, IC₂ and IC₃). Subgroup IC₁ consist of two genotype, Pusa Basmati 1 and Vallabh Basmati 24 at the coefficient $J = 0.94$ and subgroup IC₂ consist of one genotype Pusa sugandha 5 at the coefficient $J = 0.74$. Subgroup IC₃ also consist of single genotype Vallabh Basmati 22 at the coefficient $J = 0.72$. Highest genetic similarity (93.75%) measured between Pusa-Basmati 1 and Vallabh Basmati 24, Taroari Basmati and Type 3 also closely related (92.85%), Basmati 370 and Basmati 386 lies in same group but coefficient $J = 0.70$, while least genetic similarity (38.88%) was observed between Pusa sugandha 4 and Taroari Basmati. These phenomenon could be support towards strong bias power of some of the DNA marker. Highest PIC value were observed for SSR primers RM3759 is 0.748 and RM4335 is 0.748. PIC value is reflection of allele diversity and frequency among the cultivars (Lapitan *et al.*, 2007 and Wang, 2009). The average PIC value for SSR loci was 0.3632. The number of alleles detected by microsatellite marker varied from 2 to 3 with an average of 11.36 alleles per locus, while some other workers found an average of 4.5, 14.6, 7.7, 13.0, 4.5 and 4.4 alleles per locus (Jalaluddin *et al.*, 2007, Siwach *et al.*, 2004; Brondani *et al.*, 2006; Oliveira *et al.*, 2007; Thompson *et al.*, 2007, Ghneim *et al.*, 2008 and Pervaiz *et al.*, 2009, 2010). Five markers yielded two alleles while RM3332, RM4405 and RM1067 markers produce three bands.

In this study genetic diversity of Basmati rice shows clear group of traditional basmati (Basmati 370, Basmati 386, Taroari Basmati and Type 3) and evolved Basmati (Pusa Basmati 1, Pusa sugandha 5, Vallabh Basmati 22 and Vallabh Basmati 24) rice genotypes. The results indicate significant genetic variation among the genotypes. Microsatellite assays identified a number of alleles that were shared by all the Basmati varieties.

Table. 3: List of SSR (Simple sequence repeats) primers and their sequences.

S.No.	Primers	Forward Sequences	Reverse Sequences
1	RM1003	'5GATTCTTCCTCCCCTTCGTG3'	'5TTCCTGTCTCAGAACAGGGA3'
2	RM1019	'5GTTTGAACAGTAGGACTTGT3'	5AGAACATCTCACACTTCTCT3'
3	RM3332	'5ATTCTCCTCGCTTCCTCCTC3'	'5AAAGGAGAGAGCCGAGCACTG3'
4	RM3759	'5GCCACCCACTTTGAGCTTAG3'	5GATGCTGGTGC GGATCTG3'
5	RM4098	'5CGTTTGGATGAAGAAGAAGA3'	5AGTGTTCGTTTCGGATTAGA3'
6	RM4469	'5AATTTCTCATGTTTCTTCC3'	5AGTTATTCTAAGGGAGGGAC3'
7	RM4405	'5TGAAGCAATTTGATTTTCAG3'	5GAGCTGGCCTTTATTAAGT3'
8	RM3333	'5GAAGCAAAAGCTATCGACAC3'	5GAGCTTGTGGCACCTTAC3'
9	RM4355	'5GGGATGAGAGTAGAAGGCA	'5TATATGGCAAGCCTAGCG3'
10	RM4321	'5AGAAGGAAAGGTGATGATGA3'	5CCAACGTGAGGTTTATAACA3'
11	RM1067	'5CGATCCAGAGAGAATGTCTAG3'	'5TAATACGCAAGGCAGAAGGG3'

Conclusion

Traditional varieties Basmati 370, Taroari Basmati is used as donor parent programme for developing new high yielding evolved varieties of aromatic and basmati rice under breeding programmes. In present study morphological characters showed traditional basmati (Basmati 370, Taroari Basmati, Type 3) lies in same group for date of maturity, plant height, number of tillers, panicle bearing tillers, spiklet fertility percentage, while rest of other varieties followed diverse pattern. Biochemical traits study revealed that traditional basmati rice varieties recorded strong aroma, low ASV and intermediate to low AC. Molecular markers analysis revealed close relation among traditional basmati rice varieties. Basmati-370 and Basmati-386 showed very close relation with Taroari Basmati and type-3. The evolved basmati rice varieties also showed very close genetic association. Thus such morphological, biochemical and molecular markers study will be useful for diversity and the more accurate selection and study of superior genotypes.

ACKNOWLEDGEMENT

The first author is thankful to Dr.H.S.Gaur, Vice Chancellor of the S.V.P.U.A.&T. Meerut. Author is also thankful to the Department of Agricultural Biotechnology, S.V.P.U.A. &T. Meerut. For providing research facility.

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